Paclitaxel Succinate Analogs: Anionic Introduction as a Strategy to Impart Blood Brain Barrier Permeability

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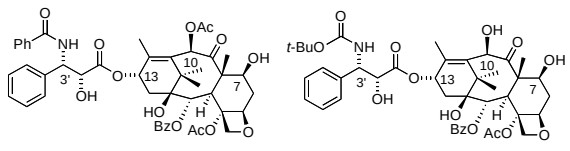
Abstract— A focused library of TX-67 (C10 hemi-succinate) analogs have been prepared including regioisomeric, functional group, and one-carbon homologs. These were prepared to investigate TX-67's lack of interaction with P-glycoprotein (Pgp). Tubulin stabilization ability, cytotoxicity, and Pgp interactions were evaluated. All carboxylic acid analogs had no apparent interactions with Pgp whereas the ester variants of the same compounds displayed characteristics of Pgp substrates. Furthermore, it is demonstrated that hydrogen-bonding properties were significant with respect to Pgp interactions. This anionic introduction strategy may allow for delivery of paclitaxel into the CNS as well as establishing a new method for delivery of other, non-CNS permeable drugs.

Paclitaxel (Taxol, **1**, Figure 1), a structurally complex natural product derived from the bark of the Pacific Yew, is one of the most studied and active anti-cancer agents known.[1,2] Although its clinical success is remarkable, the efficacy of the parent compound has limitations.[3]



1 (paclitaxel) **2** (docetaxel)

Figure 1. Paclitaxel and docetaxel

One such shortcoming is paclitaxel's inability to cross the blood-brain barrier (BBB).[4] Accordingly, paclitaxel is not an effective treatment for primary or metastatic brain cancer despite its potent anti-proliferative activity. In addition to paclitaxel's well known anti-tumor properties, it has been shown to protect primary cortical neurons from beta amyloid (Aβ)-induced cell death as well as being non-toxic to primary cortical neurons.[5] Indeed, a paclitaxel derivative that could permeate the CNS is highly desirable from both the standpoint of chemotherapy as well as an investigational therapy for neurofibrillary pathology.

A primary mechanism limiting the distribution of paclitaxel and other highly lipophillic substances into the brain is active efflux by the multidrug resistant gene product 1 (MDR1) P-glycoprotein (Pgp).[4] We have recently described a series of recognition elements required for Pgp interactions based upon the analysis of over one hundred known Pgp substrates.[6,7] This analysis revealed clusters of spatially distinct hydrogen bond acceptor units which correlated, in their relative frequency, with the strength of Pgp interaction. We have demonstrated that deletion or modification of these "recognition elements" in paclitaxel reduces interaction

with Pgp in bovine brain microvessel endothelial cells.[8] These studies also bolstered our ascertation that the C10 region of paclitaxel was particularly important for Pgp affinity.

This same examination of known Pgp substrates also revealed that compounds that carry a negative charge at physiological pH, such as those that contain a carboxylic acid, sulphonate, or nitro group, with few exceptions, *are not substrates for Pgp efflux*.[6,7] One caveat to this observation was that chemical structures that contain additional recognition elements, maintain their affinity for Pgp.

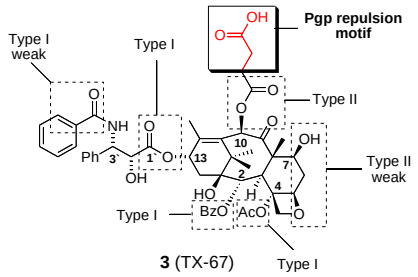
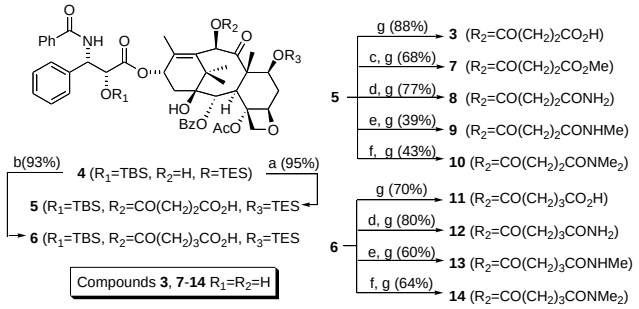


Figure 2. TX-67 recognition and repulsion elements

With this in mind we prepared a C-10 modified taxane, TX-67 (**3**, Figure 2), which only differs from paclitaxel by the addition of an acetic acid unit to the terminus of the C10 acetyl ester.[9] TX-67 (**3**) contains all of the recognition elements of paclitaxel, however, our studies suggest Pgp efflux mechanisms are substantially reduced or absent completely.[9,10] Compound **3** demonstrates improved distribution across the BBB without co-administration of Pgp inhibitors both *in vitro* and *in situ*.

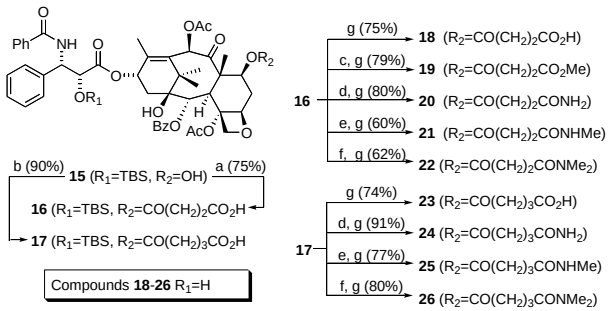
Herein we describe the synthesis and biological evaluation of a focused library of TX-67 analogs including functional group, regioisomeric, and one-carbon homologs. These were made to determine if the acid functionality was essential for Pgp evasion and if this phenomenon was restricted to only C10 analogs.



Scheme 1. Synthesis of C10 TX-67 analogs. Reagents and conditions: (a) succinic anhydride, DMAP, toluene 85 ^{o}C; (b) glutaric anhydride, DMAP, toluene 85 ^{o}C; (c) CH_2N_2, THF; (d) NH_4CO_3, THF, BOC_2O, pyridine; (e) $NH_2Me{\cdot}HCl$, EDCI, NMM, CH_2Cl_2; (f) $NHMe_2{\cdot}HCl$, EDCI, NMM, CH_2Cl_2; (g) HF-pyridine, pyridine.

The C10 functional group analogs are prepared from common intermediate **4** (Scheme 1). TX—67 (**3**) was prepared as previously descried while compounds **7-10** were accessed via the protected variant (**5**). Acid **5** is cleanly and quantitatively converted to the methyl ester via treatment with diazomethane (Scheme 1), which, after treatment with HF-pyridine solution provides methyl ester **7** in good yield. The primary amide analog **8** is prepared via mixed anhydride formation with BOC_2O followed by amide formation via ammonium bicarbonate (NH_4CO_3) under basic conditions. The N-methyl and N,N-dimethyl, amides (**9** and **10**) are generated via standard coupling procedures. The described functional group transformations were each followed by fluoride anion assisted protecting group removal. Compounds **11-14** were prepared in the same manner from glutaric acid derivative **6**.

Scheme 2. Synthesis of C7 TX-67 analogs. Reagents and conditions: (a) succinic anhydride, DMAP, toluene 85 ^{o}C; (b) glutaric anhydride, DMAP, toluene 85 ^{o}C; (c) MeOH, EDCI, DMAP; (d) NH_4CO_3, THF, BOC_2O, pyridine; (e) $NH_2Me{\cdot}HCl$, EDCI, NMM, CH2Cl2; (f) $NHMe_2{\cdot}HCl$, EDCI, NMM, CH_2Cl_2; (g) HF-pyridine, pyridine



The desired C7 analogs are accessed in short synthetic sequences from common intermediate **15** (Scheme 2). The C7 succinnic acid analog **18** is prepared by acylating the C7 hydroxyl group with succinic anhydride, giving **16**, followed by removing the 2'OTBS protecting group. The methyl ester analog (**19**) is generated from a coupling between acid **16** and MeOH followed by the same deprotection. The remaining compounds (**21-26**) were synthesized as described for the C10 analogs.

Table 1. ED_{50} ratios (compound/paclitaxel) for in vitro tubulin assembly and cytoxicity.

Entry	Compound	Tubulin Assembly	MCF7	NCI/ADR-RES
1	paclitaxel (**1**)	1.0	1.0	1.0
2	TX-67 (**3** – C10 CO_2H)	1.7	13.3	1.3
3	C10 CH_2CO_2H (**11**)	1.8	27.9	5.8
4	C7 CO_2H (**18**)	3.8	>30.0	5.8
5	C7 CH_2CO_2H (**23**)	1.0	8.8	13.6
6	C10 CO_2Me (**7**)	1.0	2.5	0.4
7	C7 CO_2Me (**19**)	1.2	0.54	0.5
8	C10 $CONH_2$ (**8**)	0.4	10	6.0
9	C10 CH_2CONH_2 (**12**)	1.8	10	5.3
10	C7 $CONH_2$ (**20**)	0.6	3.2	0.6
11	C7 CH_2CONH_2 (**24**)	0.6	5.0	6.5
12	C10 CONHMe (**9**)	0.8	7.9	6.0
13	C10 CH_2CONHMe (**13**)	1.8	2.3	>6.3
14	C7 CONHMe (**21**)	0.8	2.2	1.0
15	C7 CH_2CONHMe (**25**)	0.8	3.7	3.2
16	C10 $CONMe_2$ (**10**)	1.6	2.1	2.7
17	C10 CH_2CONMe_2 (**14**)	2.2	5.6	>6.3
18	C7 $CONMe_2$ (**18**)	1.1	10.8	0.8
19	C7 CH_2CONMe_2 (**26**)	0.7	3.9	3.0

Paclitaxel has a mean ED50 of 3.23 nM ± 1.84 and 1.53 μM ± 1.26 in the MCF-7 and NCI/ADR-RES lines, respectively.

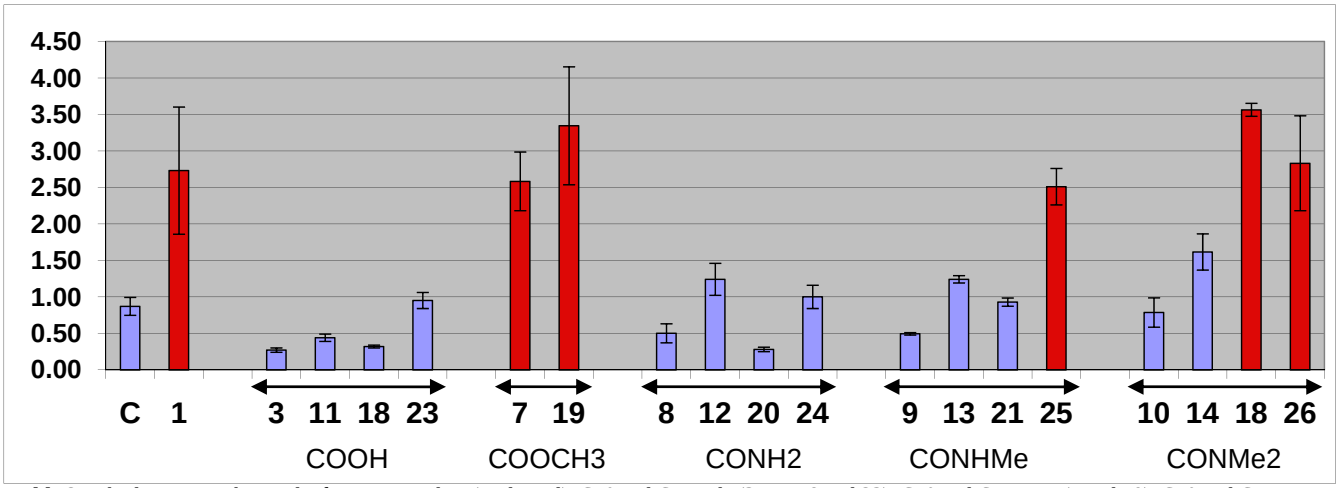


Table 2. Rhodamine uptake results for compounds **1** (paclitaxel), C10 and C7 acids (**3**, **11**, **18** and **23**), C10 and C7 esters (**7** and **19**), C10 and C7 primary amides (**8**, **12**, **20** and **24**), C10 and C7 secondary amides (**9**, **13**, **21**, and **25**), and C10 and C7 tertiary amides (**10**, **14**, **18**, and **26**) in BMEC's. Paclitaxel and the derivatives were present at a concentration of 10 μM. The concentration of rhodamine was 5μM.

All compounds were first evaluated for tubulin assembly ability as well as effectiveness against cancer cell lines (Table 1). These cell lines included the MCF7 breast cancer cell line and a drug resistant breast cancer cell line (NCI/ADR-RES).

In the tubulin assembly assay, all analogs maintained similar activity to the parent compound (entries 2-19, table 1). Against the MCF7 breast cancer cell line most analogs maintained effectiveness. The carboxylic acid analogs (entries 2-5), as a functional group class, showed the largest drop in activity (approx. 20 fold). The methyl ester variants (entries 6 and 7) maintained similar potency as compared to paclitaxel while the amides (entries 8-19) generally experienced an approximate 5-fold reduction in cytotoxicity. Versus the breast cancer cell line (NCI/ADR-RES) most analogs performed similar to paclitaxel. We had originally hypothesized an increase in effectiveness of these analogs against the MDR breast cancer cell line (MCF7-ADR [Pgp over-expressing]) however this was not the case.[11]

Our next biological screen was the rhodamine 123 uptake assay (Table 2).[12] This assay is a preliminary screen to evaluate a compounds interaction with Pgp in bovine brain microvessel endothelial cells (BMECs). In this assay, rhodamine 123 is used as a surrogate Pgp substrate. The effect of the test compound on rhodamine 123 is determined by monitoring intracellular fluorescence. If the test compound is a substrate for Pgp, then addition of it will increase rhodamine 123 uptake relative to the negative control.

All analogs containing a carboxylic acid functionality (**3**, **11**, **18** and **23)** showed no apparent interaction with Pgp. When the carboxylic acid is capped with a methyl group (**7** and **19**), in both the C7 and C10 series, a marked increase in rhodamine accumulation is observed. This supports our hypothesis that the carboxylic acid functionality is required to evade Pgp. To our surprise, many members of the amide series (**8-10**, **12-14**, **20**, **21** and **24**), did not significantly increase rhodamine accumulation. These examples are contrary to our suggestion that an anion is required and indicates that other factors are involved in recognition by Pgp. As the amide is converting from a hydrogen bond donor (**8**, **9**, **12**, **13**, **20**, **21** and **25**) to a hydrogen bond acceptor (**10**, **14**, **18** and **26**), we see transformation from a molecule that shows little interactions with Pgp to one which is an apparent substrate. This is in accord with our hypothesis that H-bond donors will not interact with Pgp and H-bond acceptors will serve as substrates. This implies that the C10 region on these analogs has a very intimate relationship with Pgp. Additionally, it is worth noting that succinate-like modifications made at the C7 position were more likely to result in an increase in Pgp interaction that the same modifications at the C10 position. This effect was substantial in the *N*-methyl amide series (**10**, **14**, **18**, and **26**) but minor in the methyl ester series (**7** and **19**). Interestingly, this trend did not hold up in the primary amide series (**8**, **12**, **20**, and **24**). Furthermore, in all cases except amides **18** and **26**, the succinic acid derivatives showed less Pgp interaction than their glutaric acid counterparts. These data further suggest that groups added to decrease Pgp interactions may be more effective at the C10 position on the paclitaxel structure.

We have prepared a focused library of paclitaxel analogs in short synthetic sequences from the parent molecule. Biological evaluation in tubulin stabilization and cytotoxicity assays demonstrate our designed analogs maintain the desired properties of paclitaxel. Our rhodamine assay indicates that the placement of a carboxylic acid functionality on either the C7 or C10 position diminishes interactions with Pgp found in bovine BBB. Since carboxylic acid transporters are known to exist in the BBB, it should be considered that the carboxylic acid functionality is a substrate for an influx pump, in a similar fashion that

paclitaxel acts as a substrate for cellular efflux. It is possible that this influx transport system is shuttling TX-67 into the cell, past the Pgp efflux system, allowing for the observed increase in BBB permeation. Current studies are underway to determine if other paclitaxel analogs containing carboxylic acid functionalities are capable of increased permeation across the BBB. Furthermore, we have illustrated that the length of linker between paclitaxel and the functional group can be modified with little effect. Unexpectedly, we have uncovered that the acid functionality *is not required in all cases* and that hydrogen bonding character of the particular analog plays a significant role. This anionic introduction strategy may allow for delivery of paclitaxel into the CNS as well as establishing a new tactic for delivery of other, non-CNS permeable drugs.

Acknowledgments

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